UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

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MEMORANDUM

Subject: Revised Risk Assessment for the new active ingredient Nitrogen Dioxide for use as a Sterilant

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NOTE: This risk assessment supercedes the previous risk assessment (DP 430487) because it includes additional exposure data submitted as MRID 49814101. This exposure data was submitted to more accurately estimate potential exposures for sterilization chamber operators.

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Executive Summary of Risk Conclusions

The risk conclusions of this assessment are as follows:

- Sterilization workers are potentially exposed to NO_2 on a daily basis over the course of their employment. The levels of concern (LOCs) are 100 ppb for 1 hour time weighted average (TWA) exposures and 34 ppb for eight hour TWAs. These LOCs are based on epidemiology studies and are applicable to short, intermediate and long term exposures.

- Sterilization worker peak (1 hour) inhalation exposures to NO_2 from opening of the isolator or sterilization chamber door(s) are not likely to be of risk concern. The maximum measured concentration during the period of highest exposure (i.e. door opening) was 177 ppb for the isolator and 158 ppb for the sterilization chamber. Given that these high exposures only occur for a few minutes during the door opening events, which occur only a few times per day, combined with the fact that the peak LOC is calculated as a one hour TWA suggest that it is highly unlikely that 1- hour exposures would exceed the LOC of 100 ppb.

- Sterilization worker 8 hour time weighted average (TWA) exposures for the isolator unit is 35 ppb, which is slightly above the LOC of 34 ppb. This exposure can be reduced to 29 ppb if the ventilation rate is increased from the label required 4 air changes per hour (ACH) to 5 ACH.

- Sterilization worker 8 hour time weighted average (TWA) exposures for the sterilization chamber is 23 ppb, which is less than the LOC of 34 ppb.

- A quantitative assessment of ecological risks from the use of NO_2 as a sterilant is not needed. Due to the use pattern of NO_2, there will be no adverse effects to non –target organisms based on a lack of exposure. Adequate safety measures will be in place to preclude any accidental exposures to non-target organisms.

1 INTRODUCTION

This memorandum addresses the human health and ecological risks associated with the use of nitrogen dioxide (NO_2) as a new pesticide active ingredient. NO_2 is intended to be used as an alternative to ethylene oxide (EtO) as a gaseous sterilant on non-critical medical devices, laboratory equipment, isolators, pharmaceuticals, and pharmaceutical equipment. NO_2 is not intended for sterile reprocessing of reusable medical devices.

For the human health risk assessment, an inhalation occupational assessment is the only potential exposure scenario since residential uses are not allowed and there is no potential for food or drinking water exposure from the sterilant use. Therefore, inhalation exposure is assessed for two exposure scenarios: 1) peak exposures to NO_2 during opening of sterilization chamber doors or isolation units after treatment; and 2) average daily exposure to NO_2 potentially released into the room air resulting from multiple cycles of treatments and opening of sterilization chamber door(s) and isolation units during a single day. This pattern of exposure may occur on a daily basis over the course of a person's employment in this occupation and is therefore considered to be long term in duration.

For ecological risk, a quantitative assessment from the use of NO_2 as a sterilant is not needed. Due to the use pattern of NO_2, there will be no potential for exposure and therefore be no adverse effects to non –target organisms. The agency believes that adequate safety measures will be in place to preclude any accidental exposures to non-target organisms.

Several sources of information were relied upon for this assessment, including open scientific literature contained in the Integrated Science Assessment published by EPA's National Center for Environmental Assessment (NCEA) in the Office of Research and Development (US EPA, 2008a) and the Technical Support Document published by the California Air Resources Board (CARB) of the California Environmental Protection Agency (California Environmental Protection Agency: Air Resources Board, 2007b). Registrant-submitted summaries as cited in MRID 49161414 were also considered.

Therefore, this antimicrobial hazard assessment relies on information from both the NCEA and CARB assessments, as well as the toxicity summary information submitted by the registrant (MRID 49161414). All of this information was used in a weight of evidence approach since each source of information provides important information to the toxicity profile of NO_2.

1.1 Tolerances and Tolerance Exemptions

EPA has not established a tolerance or tolerance exemption for residues of NO_2 in food as an active ingredient (ai) or as an intentionally-added inert ingredient in EPA-registered pesticide formulations. Also, FDA has not established a food additive regulation (FAR) or Effective Food Contact Substance Notification (FCN) for NO_2.

1.2 Proposed Use

This chemical registration is intended for the use of NO_2 as an alternative to EtO for use as a gaseous sterilant on non-critical medical devices, laboratory equipment, isolators, pharmaceuticals, and pharmaceutical equipment. Noxilizer NO_2 sterilant is intended to be used with a humidity of 75% or greater at a concentration of 20 mg/L (10640 ppm) NO_2, with a contact time of 90 minutes or greater.

2 HUMAN HEALTH RISK ASSESSMENT

2.1 Hazard Assessment

The primary exposure route of concern for the proposed use of NO_2 is inhalation. Although there is potential for dermal and ocular contact in the event of accidental release, these exposures are mitigated by the requirement for personal protective equipment (PPE) such as chemical-resistant attire, and facial protection (goggles, full-face shield, or full face respirator) that are stated on the label.

Inhalation exposures will likely occur as peak exposures to the NO_2 that is released into the workers' breathing zone during the opening of the isolator or sterilization chamber doors after a treatment cycle and from the background exposure that occurs between cycles from the NO_2 that is released from the chamber and mixes with room air. The exposure that occurs during the door opening is evaluated as a peak (one-hour) exposure to NO_2, while the daily exposure (which includes the peak exposure and the background exposure) is interpreted as an eight hour TWA. This pattern of exposure may occur on a daily basis over the course of a person's employment in this occupation and is therefore considered to be long term in duration.

Risk from inhalation exposure to NO_2 as a criterion air pollutant has been assessed by EPA's National Center for Environmental Assessment (NCEA) in the Office of Research and Development and by the California Air Resources Board (CARB) of the California Environmental Protection Agency (CAL EPA). A risk and exposure assessment for NO_2 was published by EPA's Office of Air and Radiation in 2008 (US EPA, 2008b). CARB/CAL EPA also published a Technical Support Document (CARB, 2007b) that assessed exposure and risk to NO_2. A final Integrated Science Assessment for oxides of nitrogen (including nitrogen dioxide) has recently been published by EPA's NCEA (US EPA, 2016).

Oxides of nitrogen are emitted from both gasoline-powered and diesel-powered engines and are precursors to the formation of ozone and photochemical smog. Oxides of nitrogen (including

NO_2) are assessed by EPA as criteria air pollutants under the Clean Air Act, as they have been judged by the EPA Administrator to “cause or contribute to air pollution which that may reasonably be anticipated to endanger public health or welfare;” “the presence of which in the ambient air results from numerous or diverse mobile or stationary sources.” Nitrogen dioxide is also associated with several health effects in humans, including impairment of lung host defense, airway inflammation, airway hyperresponsiveness and exacerbation of allergic asthma (US EPA, 2008a). For the 2010 review of the primary (health-based) NO_2 National Ambient Air Quality Standard (NAAQS), NCEA evaluated the health effects of NO_2 exposure using available hazard data including studies in experimental animals, controlled exposure studies in humans, and epidemiology studies of exposed populations in various cities across the United States. Epidemiology studies assessed exposures using a variety of methods, including ambient monitoring sites, monitoring at subjects’ homes or schools, and personal ambient monitoring. NCEA’s evaluation is in the 2008 Integrated Science Assessment (ISA) for Oxides of Nitrogen – Health Criteria (US EPA, 2008a). A weight-of-evidence approach was used by NCEA to determine the causality of relationships between short-term (up to 1 month) and long-term exposure (months to years) to NO_2 and an array of health effects. EPA’s Office of Air and Radiation (OAR) conducted a quantitative risk assessment for short-term exposure to NO_2 and respiratory effects using the NCEA hazard characterization. This is presented in the OAR 2008 Risk and Exposure Assessment (US EPA, 2008b). Both of these documents are comprehensive, peer reviewed assessments that consider the breadth of available data on NO_2 exposure and hazard.

NCEA’s assessment and the CARB assessment contain comprehensive information from peer reviewed scientific literature on human hazard from inhalation exposure to NO_2. These data, in combination with the summary information submitted by the registrant form a sufficient basis to assess inhalation hazard.

As the indicator for a criterion air pollutant, NO_2 has been studied extensively with respect to respiratory effects. Respiratory effects from exposure to NO_2 may be related to the ability of NO_2 to act as an oxidant. NO_2, as a reactive gas, interacts with surfactants, antioxidants, and other compounds in the epithelial lining fluid (ELF) of the lung. The compounds thought to be responsible for adverse pulmonary effects of inhaled NO_2 are the reaction products themselves or the metabolites of these products in the ELF. Lipid peroxidation is believed to be a major molecular event responsible for NO_2 toxicity, but an adverse outcome pathway has not been currently established for NO_2. In addition, the complex interactions between antioxidants, spatial differences in antioxidants between lung regions, temporal changes in antioxidant levels in response to NO_2 exposure, and species differences in antioxidant defenses are poorly understood (US EPA, 2008a).

Pharmacokinetic modeling of animal data using exposures up to 1.0 ppm indicate that humans receive approximately 2-4 times greater tissue dose of NO_2 at sensitive pulmonary sites relative to rodents (CARB, 2007b). Assuming equivalent pharmacodynamic responses between species, this would suggest that exposures as low as 250 ppb NO_2 in humans could result in the same degree of injury as exposure to 1 ppm NO_2 in rodents (California Environmental Protection Agency, Air Resources Board, 2007b). Thus, while experimental animal species may show less sensitivity to pulmonary effects of NO_2 compared to humans, animal toxicity studies can provide information on dose-response for adverse effects of NO_2 as well as the mechanistic events underlying respiratory effects observed in humans. Animal studies thus provide information that contributes to a weight of evidence determination in the assessment of pulmonary effects from NO_2 exposure, and can also provide qualitative support for the respiratory effects observed in humans.

For this assessment, human epidemiology studies that were most relevant to the exposure scenarios for the proposed use of NO_2 as a sterilant were selected, and provide the basis for selection of inhalation endpoints, with animal studies providing support in the weight of evidence. Epidemiology studies are used as the basis for endpoint selection, as the data show a strong association between exposure to NO_2 and respiratory effects, particularly the exacerbation of asthma. Epidemiology studies also provide information on responses in susceptible subpopulations.

2.1.1 Animal Studies – Acute/Short-term Exposure

Results of experimental animal studies examining adverse pulmonary effects as a result of acute/short-term exposures to NO_2 are listed in Table 1 below. These studies are cited from the CARB technical support document pages 8-35 to 8-37. Assessment of effects from acute/short-term exposures is relevant to assessing risk from peak (1 hour) occupational exposures occurring from opening of sterilization chamber doors following a sterilization cycle with NO_2. Allergic asthma responses in experimental animals provide relevant information, based in part on the string association between NO_2 exposure and exacerbation of asthma in humans. As shown in the table, at low NO_2 concentrations (<1.0 ppm), indicators of allergic asthma in antigen-sensitized animal models are not consistent in showing positive responses. However, exposure to higher concentrations of NO_2 (about 5 ppm and greater) have more consistently produced one or more indicators of allergic asthma including, enhancement of delayed-type dyspneic symptoms, increased serum IgE levels, increased pulmonary eosinophilia and epithelial injury, and increased bronchial hyperresponsiveness. This information is informative for characterizing the adverse pulmonary response from exposure to NO_2 in a weight of evidence determination.

In addition to the studies examining pulmonary effects from acute/short-term exposures in Table 1, Hine et al (1970) examined the progression of pathologic changes, relative species sensitivity, and long-term sequelae from acute exposures to NO_2. In this study, mice, rats, guinea pigs, and dogs were exposed to single doses of NO_2 ranging from 5 to 250 ppm, and exposure times from 5 minutes to 24 hours. Various physiological stressors (hot and cold temperature, exercise, co-exposure to CO_2 gas, adrenalectomy in rats) were also applied to groups of animals to evaluate the toxicologic response to NO_2 under these conditions. The authors identified a sequence of events from acute exposure ranging from minimal respiratory irritation to mortality depending on concentration and duration of exposure. At concentrations up to 20 ppm, signs of minimal irritation were observed without changes in behavior. Behavioral effects such as eye irritation, inferred from pawing and rubbing of the eyes, lacrimation and reddening of the conjunctivae were observed at 40 ppm and above. With increased concentration and duration of exposure irritation and behavioral changes were followed by increased respiration and difficulty breathing. There was also an increase in cardiac rate and terminal gasping and spasmodic respiration. Mortality was likely a result of acute asphyxia secondary to laryngeal edema and spasm or acute pulmonary edema. Mortality was observed within 60 to 480 minutes at a concentration of NO_2 as low as 75 ppm and within 30 – 120 minutes at 100 ppm. Mortality rates were high at 200 ppm and above. The authors identified the 50 ppm concentration as a dose in which mortality rarely occurred from an 8 hour exposure. Of the animals that survived the exposure period, about 20% died later from pneumonitis and secondary bacterial infection. While these effects occur at higher doses in animals and do not evaluate asthma exacerbation, these data do provide time course and dose response information that adds to the weight of evidence determination in characterizing the concentration and time dependence profile from exposure to NO_2.

Table 1 – NO_2 Animal Studies of Pulmonary Effects from Acute/Short-term Exposure

NO_2 (ppm)	Exposure	Sex	Age	Species (Strain)	Effects	Study
0.2 0.4 0.5	Continuous, 30 minutes to 7 days 20 minutes to 7 days 5 minutes to 6 days	M	Young adult	Rat (Wistar)	Qualitative increase of mast cell number in bronchi by 3 hours of exposure to 0.2 ppm. Reduction in mast cell number to normal levels by day 6 at 0.2 and 0.4 ppm. Continued increase in mast cell number through day 6 of exposure to 0.5 ppm. At 0.5 ppm: decreased histamine content ($p<0.05$) in rat trachea at 45 and 60 min time points.	1
0.5 1	4 hours 1 hour	M	Young Adult	Rat (Sprague-Dawley)	At both concentrations: Morphological changes in lung mast cells suggestive of degranulation immediately after exposure. Mast cells of exposed rats appeared normal 24-27 hour post-exposure.	2
0.5 1.0	Continuous, 12 weeks	F	4 weeks	Mouse (BALB/c)	In pre-OVA immunized and non-pre-OVA immunized mice, OVA aerosol treatment was performed at 3-week intervals during exposure. Without pre-OVA immunization: BAL fluid reductions of IgG2a at 0.5 and 1.0 ppm, and IgG1 at 1.0 ppm; BAL fluid reduction of IL-4 at 1.0 ppm; no change IL-10 or IL-12 levels. With pre-OVA immunization: BAL fluid increase in IgG1 at 1.0 ppm; no change in IgG2a, IL-4, IL-10, IL-12 levels.	3
0.7 5	2 hours/day, 3, 10 days	M F	Young Adult	Mouse (C57Bl/6)	In OVA-sensitized mice, exposure for 3 days (0.7 and 5 ppm) or 10 days (0.7 ppm) reduced OVA-induced (OVA challenge immediately before air or NO_2 exposure) BAL cellularity and eosinophil levels, and reduced histopathological evidence of OVA-induced pulmonary inflammation.	4
1.0 2.0 4.0	Continuous, 12 weeks	M NS	10 weeks 10 weeks	Rat (Wistar) Guinea pig (Hartley)	In rats: no change in number of mast cells; reduction in IgE-mediated histamine release at 2 ppm; no change in A23187-mediated histamine release. In guinea pigs: no change in number of mast cells; increased IgE-mediated histamine release at 4 ppm; no change in A23187-mediated histamine release.	5
2	24 hours	F	6-8 weeks	Mouse (BALB/c)	OVA-sensitized on days 1 and 7, challenged with aerosolized OVA on days 13 and 14, animals exposed to NO_2 prior to OVA challenge. Compared to immunized/challenged mice, NO_2 increased airway smooth muscle tone, but had no effect on percent airway eosinophils, hyperreactivity via methacholine challenge, or airway goblet cell hyperplasia.	6

NO_2 (ppm)	Exposure	Sex	Age	Species (Strain)	Effects	Study
4	2 hours/day, 5 days /week, 3 months	M F	< 1 day	Rabbit (New Zealand White)	Immunized IP with house dust mite antigen and $Al(OH)_3$ adjuvant gel, then exposed to NO_2. Compared to immunized rabbits, NO_2 had no effect on airway inflammation, airway responsiveness via histamine or methacholine provocation, or on serum IgE levels as assessed by the passive cutaneous anaphylaxis reaction.	7
4.76	4 hours/day, 5 days/week, 6 weeks	M	Young Adult	Guinea pig (Hartley)	Animals sensitized to Candida albicans IP on day 1 and week 4 of NO_2 exposure, and then challenged with C. albicans inhalation after end of exposure. NO_2 enhanced delayed-type dyspneic symptoms (tachypnea) in sensitized guinea pigs.	8
5 20	3 hours	M	6-7 weeks	Mouse (BALB/c)	OVA-immunized mice intranasally challenged with OVA just before NO_2 exposure. At 5 ppm compared to OVA-air controls: No effect on broncho-pulmonary hyperresponsiveness, epithelial permeability, neutrophilia, serum IL-4, and serum IgE. Reduced eosinophilia, serum IL-5 and mucosal metaplasia. Increased serum IgG1. At 20 ppm compared to OVA-air controls: increased broncho-pulmonary hyperresponsiveness, epithelial permeability, neutrophilia, and serum IL-5. No effect on eosinophilia, serum IL-4, or serum IgE and IgG1 levels. Reduced mucosal metaplasia.	9
5 25	6 hours/day for 1, 3, or 5 days	NS	NS	Mouse (C57BL/6)	Exposure to 25 ppm alone for 3 days increased airway hyperresponsiveness (AHR) following methacholine challenge. In mice immunized and challenged with OVA, 25 ppm caused marked augmentation of eosinophilic inflammation and terminal bronchiolar lesions. At 20 days post cessation of 5-day 25 ppm mice, eosinophilic and neutrophilic inflammation, pulmonary lesions, and AHR still present. 5 ppm NO_2 elicited no pathological findings over that produced by sensitization and challenge by OVA alone.	10
5	3 hours	F	7 weeks	Rat (Brown Norway)	Immunized IP with HDM antigen and Bordetella pertussis adjuvant, then challenged 2 weeks later with intratracheal injection of antigen NO_2 exposure after both immunization and challenge: increased serum IgE, local IgA, IgG and IgE compared to air controls; increased inflammatory cells in lungs and lymphocyte responsiveness to antigen in spleen and mediastinal lymph nodes. Single NO_2 exposures after either phase: variable responses but no trend toward suppression or enhancement of all immune parameters.	11
5-6	Continuous, 2 weeks	M	3 weeks	Mouse (BALB/c)	At beginning of exposure, mice were sensitized to either DNCB or TMA on skin, then challenged with same solutions on ears 7 days later. Serum IgE levels collected at end of exposure were enhanced by NO_2 exposure in TMA-sensitized mice that were fed either control diet or a vitamin E-deficient diet.	12
9	6 hours/day, 12 exposures over 13 days	F	Young Adult	Guinea Pig (Hartley)	Passively sensitized by anti-benzylpenicilloil bovine gamma globulin guinea pig serum i.v. on day 7 of exposure, and then challenged by intratracheally with antigen 1 day after exposure. NO_2 decreased ciliary activity, increased tracheal eosinophilia. NO_2 plus antigen antibody treatment increased tracheal epithelial damage by activated eosinophils.	13
18	4 hours	M	Young Adult	Guinea Pig (Hartley)	Exposure enhanced bronchial hyperresponsiveness ex vivo to acetylcholine, electrical field stimulation, neurokinin A, but not to histamine. Airway influx of eosinophils and neutrophils	14

Studies Included in Table 1 (taken from CARB technical support document pages 8-35 to 8-37)

1. Hayashi and Kohno (1985); Hayashi et al. (1987), 2. Thomas et al. (1967), 3. Fujimaki et al. (1998), 4. Hubbard et al. (2002), 5. Fujimaki and Nohara (1994), 6. Hussain et al. (2004), 7. Douglas et al. (1995), 8. Kitabatake et al. (1995), 9. Proust et al. (2002), 10. Poynter et al. (2006), 11. Gilmour et al. (1996), 12. Mi et al. (2002), 13. Ohashi et al. (1998), 14. Papi et al. (1999)

2.1.2 Repeated Exposure Animal Studies

Assessment of pulmonary effects in animal studies from repeated exposure to NO_2 provides information useful for a weight of evidence determination for assessment of occupational risk from inhalation exposures that occur over the course of the work day from repeated opening of sterilization chamber doors. Relevant studies examining pulmonary system responses in animal experiments from repeated exposure to NO_2 are summarized below.

Kobayashi and Miura (1995) studied the concentration- and time-dependency of airway hyperresponsiveness to inhaled histamine aerosol in guinea pigs exposed subchronically to NO_2. In one experiment, guinea pigs were exposed by inhalation to 0, 0.06, 0.5, or 4.0 ppm NO_2, 24 h/day for 6 or 12 weeks. Immediately following the last exposure, airway responsiveness was assessed by measurement of specific airway resistance as a function of increasing concentrations of histamine aerosol. Animals exposed to 4 ppm NO_2 for 6 weeks exhibited increased airway response to inhaled histamine aerosol; airway response at 12 weeks was not determined. No effects were observed at the lower exposure levels.

In another experiment conducted in this study (Kobayashi and Miura, 1995), guinea pigs were exposed by inhalation to 0, 1.0, 2.0, or 4.0 ppm NO_2, 24 h/day for 6 or 12 weeks, and airway hyperresponsiveness was determined. Hyperresponsiveness to inhaled histamine was observed in animals exposed to 4 ppm for 6 weeks, 2 ppm for 6 and 12 weeks, and 1 ppm for 12 weeks only. The results also showed that at 1 or 2 ppm NO_2, airway hyperresponsiveness developed to a higher degree with the passage of time. Higher concentrations of NO_2 were found to induce airway hyperresponsiveness faster compared to lower concentrations. When the specific airway resistance was compared to values determined 1 week prior to initiation of the NO_2 exposure, values were increased in the 2.0 and 4.0 ppm animals at 12 weeks only. Specific airway resistance was also increased to a higher degree with the passage of time.

Rombout et al. (1986) examined the influence of concentration, exposure pattern, and length of exposure on the degree and extent of morphological alterations in the rat lung. In one experiment, groups of rats were exposed to either air (control) or 20 mg/m^3 (approximately 10ppm) NO_2 continuously for 4 weeks. Six animals within each group were sacrificed after 0, 1, 2, 4, 8, 16, and 28 days of exposure. In a second experiment, groups of male rats were exposed continuously to 1, 2.5, or 5 mg/m^3 (0.53, 1.3, or 2.6 ppm) NO_2 for 28 days. Three rats each were sacrificed after 1, 2, 4, 8, 16, and 28 days of exposure. In a third experiment, intermittent vs continuous exposure to NO_2 was compared by exposing separate groups of rats to either air or 20 mg/m^3 (approximately 10ppm) once for 6 hours, 6 hours per day for 28 days, or 24 hours per day for 28 days.

Rats in the first experiment began to show morphological changes in the lung (hypertrophy and hyperplasia) after 2 and 4 days of exposure to 10 ppm NO_2, respectively. Reversal of these changes was observed after 4 and 16 days from cessation of exposure. Shortening and loss of cilia also occurred in exposed rats at day 1 of exposure. After 8 days cessation of exposure, cilia were normal again. In the second experiment, changes were noted in the 2.6 ppm exposure group, and consisted of slight thickening of the centroacinar septa and minor increases in alveolar macrophages after 2 and 4 days of exposure. From day 4 of exposure onward, loss of cilia and abnormal cilia were seen in the trachea and main bronchi. At days 16 and 28 of exposure, all epithelial cells were hypertrophied. Results of the third experiment showed that a single dose exposure to 20 mg/m^3 (10 ppm) NO_2 produced shortening, focal swelling, and loss of cilia, but complete recovery by day 4 after exposure. The study also demonstrates a fairly rapid recovery from the adverse morphological effects following cessation of exposure.

Animal data provide experimental support in characterizing the toxicological response of NO_2 from repeated exposures, which is dependent upon both the duration of exposure and concentration of NO_2. Epidemiology studies are also available for NO_2 and are included below as part of a weight of the evidence. While animal data provide evidence of the type(s) of adverse effects from exposure to NO_2 and the concentration and time-dependency of the effects, data from epidemiology studies provide relevant information for adverse effects occurring at environmental concentrations in humans and are relevant for establishing endpoints for points of departure for the inhalation risk assessment for the proposed sterilization use. The epidemiology studies also evaluate subpopulations, which includes asthmatics and children, who are likely susceptible subpopulations to the effects of NO_2. A summary of relevant epidemiology studies is presented below.

2.1.3 Epidemiology Studies in Humans

The agency's Office of Air Quality Planning and Standards (OAQPS) and the National Center for Environmental Assessment (NCEA) in 2008 developed an Integrated Science Assessment (ISA) and a Risk and Exposure Assessment (REA) in support of the development of primary and secondary National Ambient Air Quality Standards (NAAQS) for NO_2. Nitrogen dioxide is classified by the agency as a criterion air pollutant under the Clean Air Act, that is, a pollutant considered harmful to public health and the environment. These assessments were developed as part of the required 5-year time frame for review of the science and criteria used to establish NAAQS. In order to develop NAAQS for NO_2, OAQPS and NCEA reviewed and summarized data on sources of NO_2 exposure, and evidence of adverse effects from NO_2 exposure in experimental animal studies, studies in humans under conditions of controlled exposures to NO_2, and epidemiologic studies of human populations exposed to NO_2 in cities across the United States. The ISA and REA determined the spectrum of effects observed from NO_2 exposure which includes emergency department visits and hospitalizations, respiratory symptoms, airway hyperresponsiveness, airway inflammation, and effects on lung function. The ISA, using Bradford-Hill criteria, then determined the strength of association between NO_2 exposure and each described effect. Of the effects observed from NO_2 exposure, it was determined that the evidence was sufficient to infer a likely causal relationship between short-term NO_2 exposure and adverse effects on the respiratory system, specifically, exacerbation of asthma symptoms. The relationship between short-term NO_2 exposure and adverse effects on the respiratory system is supported by a large body of recent epidemiologic evidence as well as findings from human and animal experimental studies. The epidemiologic and experimental studies support positive associations between exposure to NO_2 and the respiratory effects mentioned above. For each of these effects, the 2008 REA discusses the data in support of the associations. Exposures are based on data collected from community based ambient monitoring, as well as measurement of NO_2 indoors.

Relevant epidemiology studies that show respiratory-related effects from short-term exposure to NO_2 are summarized in Table 2 below. These studies are also presented in Table 5.4-1 of the NCEA ISA (US EPA, 2008a). The data represent associations of NO_2 exposure with both acute (1-hour) and short-term (24 hour) averaging times of NO_2 concentrations in ambient air. Monitoring data for ambient levels of NO_2 are based on a State and Local Monitoring Air Stations Network of about 500 sites near urban areas across the U.S.

The data provided by the NCEA ISA in Table 2 suggest that short-term NO_2 exposure is associated with increased airway responsiveness, often accompanied by respiratory symptoms, particularly in children and asthmatics. The strongest evidence (as reported in NCEA 2008 ISA; US EPA, 2008a) for an association between NO_2 exposure and adverse human health effects comes from epidemiologic studies of respiratory symptoms and emergency department (ED) visits and hospital admissions for respiratory conditions, including asthma. Some data from toxicity studies in animals in Table 1 on allergic responses, including asthmatic type responses in animal models, supports the findings of the epidemiology studies of asthmatic responses, although as noted, effects in animal studies occur at higher concentrations compared to human data.

Table 2 – Ambient NO_2 concentrations and selected effect estimates from studies of respiratory symptoms, ED visits and hospital admissions in the U.S. and Canada. (reproduced from US EPA, 2008a)

Study	Population	Avg Time	Mean (SD)	Range	Standardized %Excess Risk (95% CI)
Respiratory Symptoms					
Schwartz et al. (1994)	6 cities, U.S.	24-h avg	13 ppb (NR)	Max: 44	61.3% (8.2, 143.4) Cough Incidence
Mortimer et al. (2002)	8 urban areas, U.S.	4-h avg	32 ppb (NR)	7- 96	48% (2, 116) Morning Asthma Symptoms
Schildcrout et al. (2006)	8 North American Cities	24-h avg	17-26 ppb (NR)	NR	4.0% (1.0, 7.0) Asthma Symptoms
Ostro et al. (2001)	LA and Pasadena, CA	1-h max	68-80 ppb (NR)	20- 220	7.0% (1.0, 13.8) Cough Onset
Delfino et al. (2002)	Alpine, CA	8-h max	34 ppb (10)	8- 53	34.6% (-17.9, 122.1) Asthma Symptoms
Delfino et al. (2003)	East LA County, CA	1-h max	7.2 ppb (2.1)	3- 14	120% (-46, 2,038) Asthma Symp. Scores >1 (not statistically significant)
Linn et al. (1996)	Los Angeles, CA	24-h avg	33 ppb (22)	1- 96	-18.2% (-47.3, 27.1) Morning Symptom Score
Strak et al. (2012)	Netherlands	24-h avg	20 ppb	9-34	Decrements in FEV_1, FVC, increases in Fe_{NO}
Emergency Department Visits – All Respiratory					
Peel et al. (2005)	Atlanta, GA	1-h max	45.9 ppb (17.3)	Max: 256	2.4% (0.9, 4.1)

Study	Population	Avg Time	Mean (SD)	Range	Standardized %Excess Risk (95% CI)
Tolbert et al. (2007)	Atlanta, GA	1-h max	43.2 ppb (NR)	1.0-181	2% (0.5, 3.3)
Emergency Department Visits – Asthma					
Jaffe et al. (2003)	Cleveland and Cincinnati, Ohio	24-h avg	Cinc:50 ppb (15) Clev:48 ppb (15)	NR	6.1% (-2.0, 14.0)
Ito et al. (2007)	New York, NY	24-h avg	31.1 ppb (8.7)	NR	12% (7, 16)
NY State Dept. of Health (2006)	Bronx and Manhattan, NY	24-h avg	34 ppb (NR)	NR	6% (1, 10) Bronx. -3% (-18, 14) Manhattan
Peel et al. (2005)	Atlanta, GA	1-h max	45.9 ppb (17.3)	NR	2.1% (-0.4, 4.5) All Ages. 4.1% (0.8, 7.6) 2-18 yrs
Tolbert et al. (2000)	Atlanta, GA	1-h max	81.7 ppb (53.8)	5.4, 306	0.7% (-0.8, 2.3)
Hospital Admissions – All Respiratory					
Burnett et al. (1997a)	16 Canadian Cities	1-h max	35.5 ppb (16.5)	NR	-0.3% (-2.4, 1.8) adjusted for CO, O_3, SO_2, CoH
Yang et al. (2003)	Vancouver, BC	24-h avg	18.7 ppb (5.7)	NR	19.1% (7.4, 36.3)<3 yrs. 19.1% (11.2, 36.3) >65 yrs
Fung et al. (2006)	Vancouver, BC	24-h avg	16.8 ppb (4.3)	7.2, 33.9	9.1% (1.5, 17.2)
Burnett et al. (2001)	Toronto, ON	1-h max	44.1 ppb (NR)	Max: 146	13.3% (5.3, 22.0)
Luginaah et al. (2005)	Windsor, ON	1-h max	38.9 ppb (12.3)	NR	6.7% (-5.4, 20.4) female. -10.3% (-20.3, 1.1) male
Hospital Admissions – Asthma					
Linn et al. (2000)	Los Angeles, CA	24-h avg	3.4 ppb (1.3)	NR	2.8% ± 1.0%
Lin et al. (2004)	Vancouver, BC	24-h avg	18.7 ppb (5.6)	4.3, 5.4	45.3% (12.7, 88.3) Boys. 23.0% (-11.7, 70.2) Girls
Lin et al. (2003)	Toronto, ON	24-h avg	25.2 ppb (9.04)	3.0, 82.0	18.9% (1.8, 39.3) Boys. 17.0% (-5.4, 41.4) Girls
Burnett et al. (1999)	Toronto, ON	24-h avg	25.2 ppb (9.1)	NR	2.60% (0, 5)

2.1.4 Toxicological Endpoints for Human Health Risk Assessment

As previously described, peak inhalation exposures will likely occur during the opening of the isolator or sterilization chamber doors after a treatment cycle and from the background exposure that occurs between cycles from the NO_2 that is released from the chamber and mixes with room air. The exposure that occurs during the door opening is evaluated as a peak (one-hour) time weighted average (TWA), while the daily exposure (which includes the peak exposure and the background exposure) is interpreted as a daily average (eight-hour) TWA. This pattern of exposure is anticipated to be repeated on a day to day basis over the course of a sterilization worker's employment and is therefore considered to be long term in duration.

Endpoints for risk assessment based on human epidemiology data are protective since responses in susceptible subpopulations (children and asthmatics) are included. The inhalation endpoints are listed in Table 3. The uncertainty factors for risk assessment may be reduced based on the use of human epidemiology data as the basis for the endpoint of concern. Therefore, the interspecies factor (extrapolation of animal to human) is reduced to 1x. The intraspecies variation factor (variability within the human population) may also be reduced to 1x as the endpoint of concern selected for the acute and daily TWA inhalation hazard are conservative, representing responses in the susceptible subpopulations of concern (asthmatics and children). The total uncertainty factor for risk assessment is therefore 1x.

Table 3 - Toxicological Endpoints for Assessing Inhalation Exposures to Nitrogen Dioxide

Exposure Scenario	Dose used for risk assessment	Uncertainty Factor(s)	Study and Toxicological Effects
Peak (1 hour TWA)	100 ppb	1	Ostro et al. (2001) and Linn et al. (2000; asthma-related effects)
Daily Average (8 hour TWA)	34 ppb	1	Strak et al. (2012); Delfino et al. (2002) (increase in respiratory symptoms)

Peak (One Hour TWA) Level of Concern

The peak (1 hour) level of concern of 100 ppb is based upon the studies of Linn et al., 2000 and Ostro et al., 2001.

In the Linn et al., 2000 and Ostro et al., 2001 studies, the 98th and 99th percentile 1-h daily maximum concentrations of measured NO_2 were 178 and 197 ppb [Linn et al., 2000], and 180 and 210 ppb [Ostro et al., 2001]) associated with increased airway responsiveness from short-term exposures (US EPA 2008b). However, because these studies did not examine responses in severe asthmatics (who could experience a more severe response), and based on published data showing NO_2 induced airway hyperresponsiveness from short-term exposure at levels below 200ppb, a value of 100ppb is selected. This value is consistent with the lower end of the range of values identified by OAQPS in the Risk and Exposure Assessment (US EPA, 2008b) at which airway hyperresponsiveness is observed from short-term exposures to NO_2 and is considered conservative.

Daily Average (8 Hour TWA) Level of Concern

The daily average (as an 8 hour TWA) level of concern of 34 ppb is based on epidemiology studies of Strak et al. (2012) and Delfino et al. (2002). Strak et al. (2012) examined pulmonary responses in 31 healthy adults after seven visits to various outdoor locations for a five hour period in each visit, where air sampling for NO_2 and other pollutants was made. Lung function [FEV_1 (forced expiratory volume in 1 sec), FVC (forced vital capacity), $FEF_{25–75}$ (forced expiratory flow at 25–75% of vital capacity), PEF (peak expiratory flow)] and fractional exhaled nitric oxide (FE_{NO}) were measured before exposure and at three time points (0, 2hr, 18hr) after exposure**.** Decrements in lung function and increases in fractional exhaled nitric oxide (an indicator of inflammation) were observed from the study group where the maximum measured concentration of NO_2 was 34 ppb. Evidence of association with NO_2 exposure is supported by the strong exposure assessment with measurement of NO_2 at the locations of the adults' outdoor exposures. Biological plausibility is provided by evidence of airway responsiveness and allergic inflammation in adults with asthma from NO_2 exposure and from animal models of allergic disease induced by NO_2 exposures in the range of 30 minutes to 6 hours.

Delfino et al (2002) also observed statistical significance for respiratory symptoms in a group of children not taking anti-inflammatory medication where the maximum 8 hour NO_2 concentration measured was 34 ppb (range 8 – 53 ppb). The level of concern selected (34 ppb) is similar to the value of 30 ppb selected by the CARB as the annual average standard and the existing annual NAAQS standard (53ppb) for NO_2, based on similar effects (increased asthma symptoms and medication use as well as emergency room visits and hospitalization for asthma, particularly in children).

2.2 FQPA Considerations

Under the Food Quality Protection Act (FQPA), P.L. 104-170, which was promulgated in 1996 to amend the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and the Federal Food, Drug and Cosmetic Act (FFDCA), under section 408(b)(2)(C), "In establishing, modifying, leaving in effect, or revoking a tolerance or exemption for a pesticide chemical residue", the Agency is directed to ensure that "there is a reasonable certainty that no harm will result to infants and children from aggregate exposure to the pesticide chemical residue." The Act further states that in the case of threshold effects, for purposes of providing this reasonable certainty of no harm, "an additional tenfold margin of safety for the pesticide chemical residue and other sources of exposure shall be applied for infants and children to take into account potential pre- and post-natal toxicity and completeness of the data with respect to exposure and toxicity to infants and children. Notwithstanding such requirement for an additional margin of safety, the Administrator may use a different margin of safety for the pesticide residue only if, on the basis of reliable data, such margin will be safe for infants and children."

The Noxilizer (NO_2) Sterilant pesticide product is not subject to a tolerance or tolerance exemption under the proposed use. Therefore, FQPA considerations do not apply to the human health assessment for this use.

2.3 Dietary Exposure and Risk

2.3.1 Dietary (Food)

The proposed label for Noxilizer NO_2 Sterilant indicates that NO_2 is not intended for direct or indirect food use. Therefore, a dietary exposure and risk assessment is not needed for the proposed use.

2.3.2 Dietary (Drinking Water)

There are no drinking water exposures from the proposed use of the Noxilizer NO_2 Sterilant product. The product is a gas that is treated using a scrubber system after sterilization and then vented to the ambient air. Therefore, a drinking water assessment is not needed.

2.4 Residential Exposure and Risk

There is potential for non-occupational exposure to NO_2 from release of NO_2 into the atmosphere from the sterilant use. However, this assessment does not quantify ambient air exposures for emissions of NO_2 into the atmosphere from the proposed use. Under existing regulations [40 CFR 52.21(m)(1)(i)(a) and 40 CFR 51.166(m)(1)(i)(a)], an ambient air quality impact analysis is required for “each pollutant that [a source] would have the potential to emit in significant amounts.” Under existing regulations [40 CFR 52.21(b)(23) and 40 CFR 51.166(b)(23)], the applicable significant emissions rate for NO_2 is 40 tons per year (tpy). This regulation is interpreted to mean that an ambient impact analysis is not necessary for pollutants with emissions rates below the significant emissions rates in paragraph (b)(23) of the regulations. For the proposed pesticidal use of NO_2, information was submitted showing emissions well below the 40 tpy threshold; therefore, the ambient air impact analysis is not necessary.

2.5 Occupational Exposure

The label indicates that “Noxilizer NO_2 Sterilant may be used only in accordance with the instruction in the Noxilizer NO_2 Sterilant Instruction Manual”. The label also requires that a calibrated NO_2 detector must be present within 5 feet of the equipment and should alarm at no more than 1 ppm. This alarm is intended to warn workers of NO_2 leaks.

Nitrogen dioxide (NO_2) will be pumped into isolation units to sterilize the inside surfaces of the units and to sterilize materials that are placed into sterilization chambers. Based on information provided by the registrant in a pre-submission meeting of October 13, 2011, the isolation units will have a typical volume of one cubic meter (1000 liters). Based on the proposed label and associated user manual, the sterilization chamber will have a volume of 360 liters. At the end of the cycle, the isolation unit or sterilization chamber is aerated with room air three to seven times until the NO_2 level inside the chamber reaches 0.5 ppm and then the unit or chamber doors are opened. The spent NO_2 from the isolation unit or sterilization chamber treatments is treated using scrubber systems that reduce NO_2 levels in the exhaust air to less than 1 ppm.

As mentioned previously, the application rate inside the isolation unit or sterilization chamber is 10,640 ppm and the required contact time is 90 minutes.

2.5.1 Occupational Exposure Data

Isolator Unit Exposure Study (MRID 491614-16)

To evaluate potential inhalation exposures during the use of Noxilizer in an isolator unit, a simulated operator exposure study (MRID 491614-16) was conducted using NO_2 in a 750 liter Class 3 Isolator unit. The isolator unit was set up in a 1930 cubic foot glovebox enclosure within a room to eliminate exposures to persons conducting the test. The front face of the isolator was 24 inches inside the front wall of the enclosure and the rear face of the enclosure was 102 inches from the rear wall of the enclosure. This equipment was operated and controlled from outside the enclosure with tygon tubing connecting the measurement instrumentation to 2 ports located in the isolator unit and 7 ports located outside the isolator unit but in the enclosure. The outside ports 1 to 6, which were 18 to 22 inches from the front and rear sides of the isolator unit, were used to measure leakage during a run and port 7, which was located 3 inches from the front hatch, was used to measure release during hatch opening. The gloves of the enclosure were near the front hatch so that the hatch could be opened without entering the enclosure.

The airflow between the enclosure and the rest of the area was initially closed during the evaluation of ports 1, 2 and 3 of day 1. It was then set at 61-63 cubic feet per minute (CFM) for ports 4 through 7 of Day 1. On day 2, it was initially set to 45 CFM during the evaluation of ports 2 and 7. It was then set to 61.5 CFM during the evaluation of all other ports of Day 2. Given that the enclosure had a volume of 1930 cubic feet, the air change rate was 1.9 air changes per hour (ACH) when the airflow was 62 CFM and 1.4 ACH when the airflow was 45 CFM. Two runs were conducted: one with an empty isolator unit and one with the isolator unit loaded with a simulated load of materials that would be sterilized. The isolator unit was dosed with a target dose of 11,440 ppm NO_2 (10 percent greater than the application rate to allow for operator inaccuracy) and maintained at an over-pressure of more than 1.0 inches of water to represent worst case conditions (the maximum allowable leakage rate for a class 3 isolator unit is 0.6% per hour).

The NO_2 levels inside the isolator unit were measured with a California Analytical Instruments Series 600 Fourier Transform Infrared Spectrometer (FTIR) instrument. The NO_2 levels were maintained at the target concentration for approximately 260 minutes during the empty run and 210 minutes during the loaded run. The maximum NO_2 levels inside the isolator unit were 12,300 ppm for the empty run of and 12,900 ppm for the loaded run. The average NO_2 levels measured during these runs were 10,900 ppm and 11,000 ppm. At the end of the run, the NO_2 was removed from the isolator unit and vented out using room air until the levels reached 0.5 ppm inside the isolator unit.

Leak testing was conducted during the run for thirty minutes at each of the six points around the outside the isolator unit. This testing was done using a Thermo Scientific Teco 42i Low Source Chemiluminescence Analyzer. This instrument has a lower detectable limit of 0.4 ppb and it had been calibrated to a range of 50 to 1000 ppb. As shown in Table 4, the individual NO_2 measurements at each port ranged from 11 ppb (minimum) to 147 ppb (maximum) during the empty run and 33 ppb (minimum) to 81 ppb (maximum) during the loaded run. The average NO_2 measurements at each port ranged from 15 ppb to 117 ppb during the empty run and 36 ppb to 68 ppb for the loaded run. The overall average for ports 1 through 6 was 76 ppb for the empty run and 49 ppb for the loaded run.

At the completion of the run, when the levels inside the chamber had decreased to 0.5 ppm, the front hatch was opened and measurements were made at the sampling port 7 just below the hatch opening for five minutes using the Chemiluminescence Analyzer. After the empty run, the NO_2 levels were 134 ppb upon hatch opening and decreased to 103 ppb after five minutes. After the loaded run, the NO_2 levels were 201 ppb and decreased to 153 ppb after five minutes.

Table 4 – NO_2 Air Concentrations during the Isolator Unit Exposure Study

Sampling Location	Number of Measurements	NO_2 in Air (ppb)					
		Minimum	Median	Average	SD	RSD	Maximum
Day 1, with an Empty Isolator Unit							
Port 1	13	11	14	15	3.2	22	23
Port 2	11	80	106	105	11	11	115
Port 3	15	110	118	117	4.4	3.8	124
Port 6	18	65	89	95	26	27	147
Port 4	16	61	63	63	0.9	1.4	65
Port 5	19	53	63	64	7.9	12	85
Average (Ports 1 through 6)				**76**			
Port 7	1 (for 5 min.)	103	119	N/A	N/A	N/A	134
Day 2, with a Full Isolator Unit							
Port 2	7	49	63	61	73	14	73
Port 3	11	56	63	68	81	14	81
Port 1	30	42	47	48	54	8.9	54
Port 4	28	35	39	39	43	6.1	43
Port 5	30	38	44	42	45	3.3	45
Port 6	31	33	35	36	40	4.1	40
Average (Ports 1 through 6)				**49**			
Port 7	1 (for 5 min.)	153	177	N/A	N/A	N/A	201

*SD = Standard Deviation, RSD = Relative Standard Deviation

Sterilization Chamber Exposure Study (MRID 49745701)

In response to AD's request for additional data to confirm the results of the isolator unit exposure study, an exposure study was performed using an RTS-360 sterilization chamber. A total of six runs (i.e. sessions) were performed with an NO_2 dose of 20 mg/liter (10,640 ppm). Three sessions were conducted with no load in the chamber and three sessions were conducted with a simulated maximum load. All sessions were performed using six aeration pulses after the sterilization pulse. The total duration of each session was approximately 60 minutes.

FTIR measurements were taken inside the chamber during the sterilization pulse and during the aeration pulses. These measurements indicated that the NO_2 concentration was 10,640 ppm during the sterilization pulse and that the NO_2 concentration was reduced to less than 500 ppb no later than after the second aeration pulse.

Electrochemical cell (EC) instrument measurements were taken with an instrument that had an operating range of 0 to 10 ppm. This instrument had been zeroed with dry air. The sampling tube of the instrument was positioned approximately six inches outside the sterilization chamber door and five feet above the ground to simulate the operator's breathing zone. The NO_2 measurements began 10 minutes prior to the start of the session and continued for ten minutes after the door was opened at the conclusion of each session. The sampling tube was manually moved to accommodate the swing of the chamber door during opening and then it was allow to fall back into place in front of the open chamber once the door was opening.

The results indicated that NO_2 levels were less than the limit of detection (100 ppb) and did not increase appreciably during the sterilization pulses. After the doors were opened, the NO_2 levels sometimes spiked up to then dropped down to less than the limit of detection within a couple of minutes. The highest spikes of 100 ppb occurred during the first and second sessions with load. A lower spike of 50 ppb occurred during the second empty session and no spikes occurred during the first and third empty sessions and the third loaded session.

Additional Sterilization Chamber Exposure Study Using a Chemiluminescent Analyzer (MRID 49814101)

Because the limit of detection of 100 ppb in sterilization chamber exposure study was above the LOC of 34 ppb for 8 hour TWA exposures, an additional sterilization chamber exposure study was done using a Chemiluminescent analyzer which is more sensitive than the EC cell that was used for the original study. The additional study was done using an RTS-360 Sterilization chamber operated in a similar manner as the original study. A total of three sessions were performed with an NO_2 dose of 20 mg/liter (10,640 ppm). All three sessions were conducted with a simulated maximum load and six aeration pulses after the sterilization pulse. The total duration of each session was approximately 90 minutes.

To remove variability caused by NO_2 in ambient air, the room used for the additional study was modified to draw makeup air from an adjacent room rather than from the facility ventilation system. This allowed the results to be corrected for ambient levels of NO_2. The ventilation modification also reduced the air exchange rate to 1.5 air changes per hour.

NO_2 measurements were taken with a California Analytical Instruments 600 Series Chemiluminescent NO_x Analyzer. This instrument has an operating range of 0 to 3 ppm NO_2 and a resolution of 10 ppb NO_2. It had been calibrated to an LOD of 12 to 21 ppb NO_2 based on 3X the standard deviation of 4 to 7 ppb for a factory run of a demo instrument on ambient air for several hours. The sampling tube of the instrument was positioned approximately six inches outside the sterilization chamber door and five feet above the ground to simulate the operator's breathing zone. The NO_2 measurements began 10 minutes prior to the start of the session and continued for ten minutes after the door was opened at the conclusion of each session. The sampling tube was manually moved to accommodate the swing of the chamber door during opening and then it was allow to fall back into place in front of the open chamber once the door was opening.

The results were reported as the NO_2 air concentrations measured near the sterilizer during the session minus the NO_2 air concentrations measured in the makeup air. The results indicated that NO_2 levels were less than the limit of detection (21 ppb) and did not increase appreciably during the sterilization pulses. After the doors were opened during sessions 1 and 2, the NO_2 levels spiked up to138 and 44 ppb, respectively, then dropped down to the LOD in approximately 20 seconds. The NO_2 level did not increase above the LOD when the door was opened after session 3.

2.5.2 Occupational Risk Assessment

Occupational exposure will consist of peak exposures from the NO_2 that is released from the sterilization chamber or isolator unit during single door opening events into the workers' breathing zone, and 8-hour average exposure that consists of the peak exposures combined with background exposures that occur between door opening events. The background exposures are to the amount of NO_2 that leaks out of the isolator unit during the sterilization cycle and to the amount of NO_2 that is released from the sterilization chamber during door opening that is mixed with the room air. The label requires a contact time of 90 minutes (i.e. 1.5 hours) at the application rate of 10,640 ppm. Given this time period and allowing another hour for ramp up prior to treatment and aeration after treatment combined with the time to change out loads yields a total cycle time of 2.5 hours. If it is assumed that one operator would operate two sterilization chambers, then it is anticipated that a door opening event would occur every hour and 15 minutes which would yield approximately 6 door opening events per 8 hour workday.

<u>Exposure Assessment Based on the Isolator Unit Exposure Study</u>

Based on the isolator unit exposure study, which indicated that NO_2 levels during door opening after a loaded run ranged from 201 to 153 ppb (Median = 177 ppb) for a five minute period, and assuming that this exposure would occur once per hour between treatment cycles, it is anticipated that one hour TWA exposures to NO_2 would be 60 ppb as shown in Table 3. This exposure does not exceed the one hour TWA LOC of 100 ppb and is therefore not a risk concern.

As shown in Table 5, the 8 hour TWA exposure is 57 ppb when the peak exposure that occurs during hatch opening events is included with the background exposure of 49 ppb that occurs between hatch opening events. The exposure study was conducted in an enclosure that had a 24 inch clearance on the front side to allow for glovebox operation, and it is anticipated that an actual NO_2 treatment facility would have more clearance on the front side and thus exposures would be lower. In addition, the Noxilizer System Operator Manual Model #RTS 360, which is referenced on the label, indicates that a Noxilizer NO_2 treatment facility is required to have a ventilation rate of at least 4 ACH which is approximately two times greater than the 1.4 to 1.9 ACH that was maintained during the loaded run of the exposure study. If the ventilation rate is 4 ACH (as required on the label), then the exposures will be proportionately reduced. Thus, background NO_2 air concentrations would be reduced from 49 ppb to 25 ppb and the daily 8 hour TWA is reduced to 35 ppb. This TWA exceeds the LOC of 34 ppb for 8 hour exposures. Increasing the ventilation rate from 4 ACH to 5 ACH would result in an 8 hour TWA of 29 ppb, which would not exceed the daily average LOC of 34 ppb and would not be a risk concern.

Table 5 – NO_2 Exposures Estimated from the Isolator Exposure Study

Ventilation Rate (ACH)	Peak Exposure (ppb)	Peak Exposure Period (Minutes/Day)	Background Exposure[G] (ppb)	Background Exposure Period (Minutes/Day)	One Hour TWA[K] (ppb)	8 Hour TWA[L] (ppb)
1.4 to 1.9[A]	177[D]		49[H]		60	57
4.0[B]	177[E]	30[F]	25[I]	450[J]	38	35
5.0[C]	177[E]		19[I]		32	29

A. The ventilation rate for the exposure study was 1.4 to 1.9 Air Changes per Hour (ACH).
B. A ventilation rate of 4.0 ACH is required by the Noxilizer System Operator Manual.
C. Back calculated to yield an 8 hour TWA of less than the LOC of 34 ppb.
D. Based on the exposure study for the loaded run immediately after hatch opening.
E. Peak exposure is not affected by the ventilation rate.
F. Assuming 5 minutes peak exposure per door opening event and six door opening events per day.
G. Represents the exposure that occurs between door opening events.
H. Based on the simulated exposure study for the loaded run.
I. Adjusted for the ventilation rates.
J. Based on an eight hour workday (480 minutes) minus the peak exposure period (30 minutes)
K. TWA = [(Peak Exp. (ppb) * 5 minutes/hour) + (Background Exp. (ppb) * 55 minutes/hour)]
60 minutes per hour
L. TWA = [(Peak Exp. (ppb) * 30 minutes/day) + (Background Exp. (ppb) * 450 minutes/day)]
480 minutes per day

Exposure Assessment Based on the Sterilization Chamber Exposure Study

The additional chamber exposure study (MRID 49814101) indicated that the peak exposures during door opening events was a maximum of 158 ppb and these exposures lasted for less than one minute. The background exposures between door opening events was less than the limit of detection (LOD) of 21 ppb. Assuming an exposure frequency of six door opening events per day (based on the cycle times) and a peak exposure duration of one minute per event (based on MRID 49814101), the resulting one and eight hour TWAs are both 23 ppb as shown in Table 6. The one hour TWA is less than the LOC of 100 ppb and is not of concern. The eight hour TWA of 23~~2~~ ppb is less than the LOC of 34 ppb and is not of concern.

Table 6 – NO_2 Exposures Estimated from the Sterilization Chamber Exposure Study

Ventilation Rate[A] (ACH)	Peak Exposure (ppb)	Peak Exposure Period (Minutes/Day)	Background Exposure[D] (ppb)	Background Exposure Period (Minutes/Day)	One Hour TWA[F] (ppb)	8 Hour TWA[G] (ppb)
1.5 ACH	158[B]	6[C]	21	474[E]	23	23

Notes for Table 6.

A. The exposure study (MRID 49814101) was conducted in a room that was modified to minimize the influx of ambient NO_2 from outside air circulated thorough the main ventilation system.
B. Based on the maximum peak level measured occurred during door opening.
C. Assuming 1 minute peak exposure per door opening event and six events per day.
D. Represents the exposure that occurs between door opening events.
E. Based on an eight hour workday (480 minutes) minus the peak exposure period (6 minutes)

F. TWA = $\frac{[(\text{Peak Exp. (ppb)} * 1\ \text{minute/hour}) + (\text{Background Exp. (ppb)} * 59\ \text{minutes/hour})]}{60\ \text{minutes per hour}}$

G. TWA = $\frac{[(\text{Peak Exp. (ppb)} * 6\ \text{minutes/day}) + (\text{Background Exp. (ppb)} * 474\ \text{minutes/day})]}{480\ \text{minutes per day}}$

2.6 Human Health Uncertainty Analysis

Compared to controlled studies of human exposure to NO_2, where a concentration-response relationship and threshold for effect can be determined, the effects of exposure to NO_2 as reported in epidemiology studies may be confounded by the presence of other pollutants in air that may also contribute to the effects observed. That is, it is sometimes difficult to determine from epidemiology studies the extent to which NO_2 is independently associated with respiratory effects or if NO_2 is a marker for the effects of another traffic-related pollutant or mix of pollutants. Despite this, the available evidence indicates that NO_2 associations generally remain robust in multi-pollutant models and supports a direct effect of NO_2 exposure on respiratory effects. Some of these studies show associations at concentrations below the current published air quality standards for NO_2.

In addition, as noted in the Office of Air Quality Planning and Standards' document, Risk and Exposure Assessment to Support the Review of the NO_2 Primary National Ambient Air Quality Standard (US EPA, 2008b), "epidemiologic evidence for respiratory effects from NO_2 exposure is consistent, in that associations are reported in studies conducted in numerous locations and with a variety of methodological approaches (US EPA, 2008b)." The levels of concern selected in this assessment from epidemiology studies are also consistent with those determined by the CARB for NO_2 and are also consistent in the effects observed from NO_2 exposure.

The measurement of NO_2 levels in the workplace for comparison with the 8 Hour LOC of 34 ppb is complicated by the fact that NO_2 is an air pollutant released in measurable~~large~~ amounts from sources such motor vehicles and power plants. Based on data from state and local monitoring stations as reported in Tables 2-2 and 2-3 of the Integrated Science Assessment for Oxides of Nitrogen – Health Criteria (US EPA, 2016), the annual average NO_2 concentration is a mean of 8.6 ppb with a maximum of 26 ppb (n=1,041). The 1 hour daily maximum NO_2 concentration is a mean of 19 ppb with a 99th percentile value of 55 ppb (n=390,713). The highest 1 hour daily maximum mean and 99th percentile concentrations are 41 and 73 ppb in Denver (n=2184), 28 and 63 ppb in Los Angeles (n= 30,612) and 28 and 64 ppb in New York City (n=11,803).

2.7 Human Health Conclusions

Based on the weight of evidence and the information presented in this assessment, the primary concern from the proposed use of NO_2 as a gaseous sterilant is for human health, specifically adverse effects on the respiratory system from acute and short-term inhalation exposure to sterilization workers. There is strong evidence for a causal relationship between NO_2 exposure and asthma exacerbation. Both experimental studies in animals and human epidemiology data provide evidence for this health effect. Epidemiology studies provide the additional advantage of protection for susceptible subpopulations. Using information obtained from the NCEA science assessment of NO2 and animal toxicology studies from the CARB technical support document, value(s) of 100 ppb and 34 ppb for peak and daily time weighted average worker assessments have been selected. The value of 100 ppb is appropriate for the peak assessment, as this represents a 1-hour maximum concentration of nitrogen dioxide that has been associated with respiratory symptoms. The value of 34 ppb is selected for the 8 hour TWA inhalation assessment, as this value represents an 8 hour maximum in a study with asthmatic children that observed a significant association with respiratory effects.

The one hour peak exposures of sterilization workers using NO_2 in isolators or sterilization chambers are not likely to exceed the LOC of 100 ppb. This is based on the submitted chamber and isolator exposure studies, the procedures outlined in the label and in the operating manual, and the nature of the equipment used. The maximum measured concentration during the period of the highest exposure (i.e. door opening) was 177 ppb for the isolator and 158 ppb for the sterilization chamber. Given that these high exposures only occur for a few minutes during the door opening events which occur only a few times per day, combined with the fact that the peak LOC is calculated as a one hour TWA suggest that it is highly unlikely that exposures would exceed the LOC. Even if exposures during isolator operation were higher than those measured (i.e. 500 ppb instead of 177 ppb) and if the duration of exposure were longer (i.e. 10 minutes instead of 5 minutes), the resulting one hour TWA of 88 ppb for isolator operators would still be less than the LOC of 100 ppb. A similar argument could also be made for the sterilization chamber operators. It should also be noted that the label requires that an air monitoring device, with an alarm setting of 1.0 ppm (1000 ppb), to be positioned within 5 feet of the equipment. In addition, the FTIR measurements taken inside the chamber during the aeration phase indicated that the NO_2 levels dropped from the treatment level of 10,000 ppm to the limit of detection (0.5 ppm) by the end of the second pulse.

The 8 hour TWA exposure is 35 ppb for the isolator chamber scenario with a ventilation rate of 4 air changes per hour as required by the label. This TWA exceeds the LOC of 34 ppb for 8 hour exposures. Increasing the ventilation rate from 4 ACH to 5 ACH would result in an 8 hour TWA of 29 ppb, which would not exceed the daily average LOC of 34 ppb and would not be a risk concern.

The 8 hour TWA exposure is 23 ppb for the sterilization chamber scenario and does not exceed the LOC of 34 ppb for 8 hour exposures.

3 ECOLOGICAL RISK ASSESSMENT

3.1 Exposure Assessment

3.1.1 Environmental Fate Summary

Nitrogen dioxide is an inorganic substance and a gas at ambient room temperature. The aqueous photolysis processes are well understood, as well as the speciation of NO_2 in the atmosphere. Nitrogen dioxide and other nitrogen compounds do not bind with soils under aerobic or anaerobic conditions.

In the event of release into waste water, NO_2 will undergo oxidation and reduction depending on the conditions of the wastewater.

For the use of NO_2 as a fumigant, release into environmental media like water and soils is unlikely; however, an accidental release into atmosphere if it is leaked from the treatment tanks is a possibility. However, standards have been established for NO_2 in ambient air by OAR as discussed above.

The agency recognizes that NO_2 is present in the atmosphere and NO_x species are found in environmental media like air, soils and water. Nitrogen and its oxides are well known and extensively investigated and published in the scientific literature, in particular on atmospheric contaminants. The particular use scenario as a sterilant gas is not likely to add significant additional NO_2 into the atmosphere and no risk is anticipated.

3.1.2 Ecological Effects Risk Characterization

A quantitative assessment of the ecological risks from using NO_2 as a sterilant will not be necessary because of the unique way it is used to sterilize laboratory equipment, isolators, medical devices, pharmaceuticals and pharmaceutical production equipment in an enclosed sterilizer. NO_2 is produced in an enclosed generator and then pumped into the sterilization unit. This unit is completely sealed and the NO_2 is contained within the sterilizer. The sterilizers are housed in an indoor laboratory setting, which will preclude exposure to non-target organisms.

Environmental exposure can occur when the NO_2 is vented out into the atmosphere after the sterilization process has been completed. The Noxilizer equipment mitigates this potential exposure by using sodium permanganate containing scrubbers (3 primary and 2 backups) that remove most of the nitrogen dioxide during the discharge process. The scrubbers release 0.1 ppb or less of NO_2 after venting. This amount would be insignificant and well below the ambient levels of the NO_2 naturally occurring in the environment. The system has safeguards in place that will ensure that accidental releases of NO_2 above 1 ppm will not occur. An alarm system is activated when 1 ppm or more of NO_2 is released into the environment and the venting system will be shut down. This system of scrubbers and alarms will ensure that the amount of NO_2

released into the environment will be minimal. Therefore, the amount of NO_2 released into the environment will not be high enough to cause risk to non-target organisms. To ensure that these safety procedures are followed, the chemical may only be used with Noxilizer produced or licensed equipment and can only be applied via a closed system.

Due to the restricted use pattern of Noxilizer NO_2 Sterilant, NO_2 will not cause any adverse effects to non-target organisms based on a lack of exposure. The registrant has satisfactorily shown that this product will be contained in the sterilization equipment and there are adequate safety measures in place to preclude accidental exposure to non-target organisms. Therefore, all aquatic, avian, and terrestrial plant ecological effects studies have been waived.

3.1.3 Threatened and Endangered Species

There is no reasonable expectation for the registered use of NO_2 to cause direct or indirect adverse effects to threatened and endangered species. No adverse modification of any designated critical habitat for such species is expected from the use of NO_2. This chemical will be contained in a sterilization chamber when it is used and will not come into contact with any threatened or endangered species. Any release of NO_2 after the sterilization process will be minimal and not present any risk to threatened and endangered species. EPA has made a “no effect” determination under the Endangered Species Act (ESA) for all listed species and designated critical habitat for such species and has therefore concluded that consultation with the Fish and Wildlife Service and the National Marine Fisheries Service under ESA section 7(a)(2) is not required.

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